Histopathology

Briefly, the paraffin tissue sections were dewaxed, hydrated, and then antigen retrieval. Subsequently, the tissue slides were incubated with primary antibodies using rabbit anti-human PD-1 polyclonal antibody (5 μg/ml, cat # PA5-20351; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA.) or mouse anti-human PDL-1 monoclonal antibody (5 μg/ml cat # 14-5983-82; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA.) at 4°C overnight, followed by incubation with secondary antibodies (cat # K5007; Dako). Staining was performed with DAB and counterstained with hematoxylin. Two senior pathologists who were blinded to the clinical data independently selected five non-overlapping and discontinuous regions to calculate the mean for statistical analysis. The numbers of PD1 and PD-L1 cells were quantified at ×400 (0.0484 mm2). Variations in the results within a range of 5% were reassessed, and a consensus decision was made. PD1 and PD-L1 positivity were considered per specimen by a 5% expression cut-off value (PD1+ and PD-L1+ tumor cells/total tumor cells); cases with expression greater than 5% were considered as PD1 and PD-L1 positive.